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Draft Genome Sequences of *Bacillaceae* Strains Isolated from the International Space Station

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ABSTRACT

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ANNOUNCEMENT

The family *Bacillaceae* is composed of mostly aerobic or facultatively anaerobic rods with a Gram-positive cell wall. Members of this group are ubiquitous in nature, found in soil, sediments, air, freshwater, marine ecosystems, and foods and in extreme environments with high temperatures (1). *Bacillaceae* groups are reported to produce a wide range of valuable and thermostable extracellular enzymes (2). The majority of *Bacillaceae* can form endospores that are crucial for survival in harsh environments (3, 4). This spore-forming property makes it important for NASA planetary protection purposes because spore-forming bacteria are more likely to survive sterilization procedures and the harsh environments of outer space, making them a potential threat for forward contamination (5).

This report presents the draft genome sequences of three species (*Bacillus amyloliquefaciens*, *Bacillus cereus*, and *Paenibacillus polymyxa*) belonging to the family *Bacillaceae*, isolated from predefined surfaces at various locations on the International Space Station. The samples were collected using premoistened polyester wipes (6), brought back to Earth, aseptically transferred to a 500-ml bottle containing 200 ml of phosphate-buffered saline, mixed by vigorously shaking, and concentrated via a filtration system. An appropriate aliquot of each sample was inoculated onto either Reasoner's 2A (R2A) (25°C; 7 days) or blood (37°C; 2 days) agar. A single colony from each incubated plate was used for genomic DNA extraction using the ZymoBIOMICS MagBead DNA kit according to the manufacturer's instructions (Zymo Research, USA). All isolates were identified by 16S rRNA sequencing based on similarity to their type strain 16S rRNA, as previously reported (6). To create the whole-genome sequences (WGS) of these strains, shotgun libraries were prepared using the Illumina Nextera Flex protocol (7), using NovaSeq 6000 S4 flow cell 2 × 150 paired-end (PE) sequencing. Verification of the quality of the raw sequencing data was carried out using FastQC v0.11.7 (8). Quality control for adapter trimming and quality filtering were performed using fastp v0.20.0 (9), and then SPAdes v3.11.1 (10) was used to assemble all the cleaned sequences. Fastp quality control was based on the following three parameters: (i) correction of mismatches in overlapped regions of paired-end reads, (ii) trimming of autodetected adapter sequences, and (iii) quality trimming at the 5' and 3' ends. To determine the quality of the assembled sequences, the number of contigs, the N_{50} value, and the total length were calculated using QUAST v5.0.2 (11). Default parameters were used for all software. The average nucleotide identity (ANI) (12) was calculated using OrthoANIu by comparing each of the 29 *Bacillaceae* scaffolds to the WGS of the respective type strains. The ANI range was 93.98% to 94.17% for *B. amyloliquefaciens*, and the ANI was 98% for both *B. cereus* and *P. polymyxa*. All other genomic statistics are given in Table 1.

TABLE 1.

Summary of draft whole-genome sequences of *Bacillaceae* strains isolated from various International Space Station environmental surfaces

Sample name	Bacterial species	WGS accession no.	SRA accession no.	Isolation location ^a	No. of contigs	Genome size (bp)	<i>N</i> ₅₀ (b
IIF7SW-P1	<i>Bacillus amyloliquefaciens</i>	JABVDW000000000	SRR11948426	Overhead 3	33	3,908,309	234,63
IIF7SW-P2	<i>Bacillus amyloliquefaciens</i>	JABVDS000000000	SRR11948402	Overhead 3	34	3,906,939	268,70
IIF7SW-P3	<i>Bacillus amyloliquefaciens</i>	JABVDR000000000	SRR11948401	Overhead 3	33	3,908,243	234,63
IIF7SW-P4	<i>Bacillus amyloliquefaciens</i>	JABVDP000000000	SRR11948399	Overhead 3	31	3,906,699	268,70
IIF7SW-P5	<i>Bacillus amyloliquefaciens</i>	JABVDV000000000	SRR11948415	Overhead 3	36	3,907,462	268,70
IIF7SW-B1	<i>Bacillus amyloliquefaciens</i>	JABVDX000000000	SRR11948427	Overhead 3	32	3,907,264	268,70
IIF7SW-B4	<i>Bacillus amyloliquefaciens</i>	JABVDQ000000000	SRR11948400	Overhead 3	22	4,214,765	1,035,3
IIF7SC-B1	<i>Bacillus amyloliquefaciens</i>	JABVDT000000000	SRR11948403	Field control	36	3,907,040	268,70
IIF7SC-B4	<i>Bacillus amyloliquefaciens</i>	JABVDU000000000	SRR11948404	Field control	35	3,907,001	268,70
IIF4SW-P2	<i>Bacillus thuringiensis</i>	JABVDN000000000	SRR11948425	Dining table	18	5,303,558	2,681,5
IIF4SW-P3	<i>Bacillus thuringiensis</i>	JABVDI000000000	SRR11948420	Dining table	18	5,302,927	2,862,9
IIF4SW-P4	<i>Bacillus thuringiensis</i>	JABVDH000000000	SRR11948419	Dining table	17	5,303,162	3,967,1

IIF4SW-P5	<i>Bacillus thuringiensis</i>	JABVDM000000000	SRR11948424	Dining table	18	5,303,374	2,863,4
IIF4SW-B1	<i>Bacillus thuringiensis</i>	JABVDJ000000000	SRR11948421	Dining table	17	5,302,884	3,967,5
IIF4SW-B2	<i>Bacillus thuringiensis</i>	JABVDG000000000	SRR11948418	Dining table	18	5,303,293	2,863,2
IIF4SW-B3	<i>Bacillus thuringiensis</i>	JABVDK000000000	SRR11948422	Dining table	18	5,303,485	2,863,4
IIF2SG-B1	<i>Bacillus thuringiensis</i>	JABVDL000000000	SRR11948423	CRV2-L2	19	5,303,842	2,682,3
IIF3SG-B3	<i>Bacillus thuringiensis</i>	JABVDF000000000	SRR11948417	CRV2-L3	18	5,303,031	2,863,3
IIF7SG-B4	<i>Bacillus thuringiensis</i>	JABVDO000000000	SRR11948398	CRV2-L7	17	5,302,496	3,967,2
IIF1SG-B5	<i>Bacillus thuringiensis</i>	JABVDE000000000	SRR11948416	CRV2-L1	18	5,303,272	2,864,9
IIF2*SW-P2	<i>Paenibacillus polymyxa</i>	JABVCY000000000	SRR11948409	WHC	34	5,788,525	1,496,2
IIF2*SW-P4	<i>Paenibacillus polymyxa</i>	JABVCV000000000	SRR11948406	WHC	30	5,790,156	1,573,8
IIF2SW-B2	<i>Paenibacillus polymyxa</i>	JABVDB000000000	SRR11948412	WHC	35	5,788,707	1,496,2
IIF5SW-B3	<i>Paenibacillus polymyxa</i>	JABVDC000000000	SRR11948413	Overhead 4	34	5,789,544	1,497,6
IIF5SW-B4	<i>Paenibacillus polymyxa</i>	JABVDA000000000	SRR11948411	Overhead 4	36	5,789,252	1,496,2
IIF8SW-P3	<i>Paenibacillus polymyxa</i>	JABVCW000000000	SRR11948407	Crew quarters	34	5,790,736	670,850
IIF8SW-P4	<i>Paenibacillus polymyxa</i>	JABVCZ000000000	SRR11948410	Crew quarters	32	5,790,548	1,573,8
IIF8SW-P5	<i>Paenibacillus polymyxa</i>	JABVCU000000000	SRR11948405	Crew quarters	33	5,789,239	1,496,1

IIF8SW-B4	<i>Paenibacillus polymyxa</i>	JABVCX000000000	SRR11948408	Crew quarters	37	5,789,049	670,850
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^aWHC, waste and hygiene compartment; CRV, commercial resupply vehicle. Hyphenated designations indicate the CRV number followed by the location.

Data availability.

The WGS and raw data have been deposited in GenBank under the BioProject accession number [PRJNA637984](#) and also in the NASA GeneLab system (<https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-303/>). The version described in this paper is the final version.

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Associated Data

This section collects any data citations, data availability statements, or supplementary materials included in this article.

Data Availability Statement

The WGS and raw data have been deposited in GenBank under the BioProject accession number [PRJNA637984](#) and also in the NASA GeneLab system (<https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-303/>). The version described in this paper is the final version.

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